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Effect of simulated microgravity on oxidation-sensitive gene expression in PC12 cells

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Abstract

Oxygen utilization by and oxygen dependence of cellular processes may be different in biological systems that are exposed to microgravity (micro-g). A baseline in which cellular changes in oxygen sensitive molecular processes occur during micro-g conditions would be important to pursue this question. The objective of this research is to analyze oxidation-sensitive gene expression in a model cell line [rat pheochromocytoma (PC12)] under simulated micro-g conditions. The PC12 cell line is well characterized in its response to oxygen, and is widely recognized as a sensitive model for studying the responses of oxygen-sensitive molecular and cellular processes. This study uses the rotating wall vessel bioreactor (RWV) designed at NASA to simulate micro-g. Gene expression in PC12 cells in response to micro-g was analyzed by DNA microarray technology. The microarray analysis of PC12 cells cultured for 4 days under simulated micro-g under standardized oxygen environment conditions revealed more than 100 genes whose expression levels were changed at least twofold (up-regulation of 65 genes and down-regulation of 39 genes) compared with those from cells in the unit gravity (unit-g) control. This study observed that genes involved in the oxidoreductase activity category were most significantly differentially expressed under micro-g conditions. Also, known oxidation-sensitive transcription factors such as hypoxia-inducible factor-2α, c-myc, and the peroxisome proliferator-activated receptor-γ were changed significantly. Our initial results from the gene expression microarray studies may provide a context in which to evaluate the effect of varying oxygen environments on the background of differential gene regulation of biological processes under variable gravity conditions.

Keywords

Rotating wall vessel; Simulated microgravity; Gene expression; Microarray

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1. Introduction

On long duration space flights, astronauts undergo many physiological changes such as loss of bone mass, muscle strength, and cardiovascular fitness, as a result of reduced metabolic activities and lower cellular and tissue oxygen demand (Freed and Vunjak-Novakovic, 2002). In order to investigate the effects of micro-g on cellular responses, simulation can be achieved through the use of bioreactors in which responses of cell cultures can be characterized under conditions of ground-based simulated micro-g. Such studies during space flight, even such as possible on the current earth orbiting space station are limited by constraints such as time, financial costs, and extensive equipment.

Suspension cell culture conditions for simulating micro-g of outer space on earth can be optimized in the laboratory using Rotating Wall Vessel (RWV) bioreactors. The RWV bioreactor, developed by National Aeronautics and Space Administration/Johnson Space Center (NASA/JSC), can be used to generate micro-g conditions for the cell suspension when it is completely filled with the culture medium (i.e., zero headspace) and aerated by a hydrophobic membrane (Schwarz et al., 1991; Hammond and Hammond, 2001; Nickerson et al., 2003). The cell culture vessel continuously rotates on a horizontal axis to the ground, and thus, the gravitational vectors are randomized over the surface of the cells. In addition, the fluid shear stress is minimized through the synchronous solid-body rotation.

This system has been used to understand and investigate the effects of micro-g on various cells and tissues (Unsworth and Lelkes, 1998; Hammond and Hammond, 2001). A number of early reports described the effects on growth and differentiation in cells cultured in the RWV bioreactors. The rat pheochromocytoma (PC12) cells cultured in the RWV showed formation of large aggregates exhibiting neuroendocrine differentiation and altered cellular signaling mechanisms (Lelkes et al., 1998). Human hepatocytes formed three-dimensional assemblies when exposed to simulated micro-g (Khaoustov et al., 1999). Cultures of PC12 and SH-SY5Y cells in the simulated micro-g resulted in high cell viability and cell aggregation (Wang and Good, 2001).

Since changes in gene expression reflect different patterns of the physiological response in cells and tissues, the understanding of which products of gene expression change exhibit and what functions these gene products serve is essential for exploring the biological impact of such changes in micro-g environments. Changes in the multigene patterns of expression can provide further clues about responses in regulatory mechanisms (Napoli et al., 2003). Micro-g environments have been shown to elicit both up- or down-regulation of specific genes in a rather wide assay of cell types. For instance, in the human osteosarcoma cell line M-63, gene expression of collagen I a1, alkaline phosphatase, and osteocalcin was reduced compared with unit-g control (Carmeliet et al., 1997). Specific transcription factors of kidney cells exposed to micro-g conditions under-went large changes, including the genes regulation of Wilms' tumor zinc finger protein and the vitamin D receptor (Hammond et al., 2000).

Several conventional techniques for the analysis of gene expression are available, such as Northern blotting, differential display, and dot blot analysis. One of the most commonly used approaches is the gene expression microarray technique, which can identify differences in the expression level of thousands of gene products (Schena et al., 1996). This microarray technique is a powerful tool for comparing gene expression in the same cell or tissue culture under different conditions. For instance, a recent study has utilized the human DNA expression microarray to investigate human liver cells (HepG2) cultured in the simulated micro-g condition (Khaoustov et al., 2001).

While many researchers are conducting oxygen transport studies in cells cultured under unit-g conditions (e.g., Vadapalli et al., 2000), little information is available on responses of oxygen-

dependent cellular processes under micro-g. The PC12 cell line and its molecular machinery is well characterized in its response to oxygen (both high and low oxygen environments), and thus this cell line is a useful model for studying the oxygen-sensitive molecular and cellular mechanisms (Greene and Tischler, 1976; Czyzyk-Krzeska et al., 1994; Kumar et al., 1998). These cells are responsive to a wide range of molecular oxygen concentration. For example, Ishiguro et al. (2001) showed oxygen loading increased the damage of PC12 cells by thenoyltrifluoroacetone (TTFA, a complex I inhibitor). In the opposite direction, Kumar et al. (1998) demonstrated that hypoxia causes the enhanced release of catecholamines from PC12 cells.

The objective of this study is to document the responses in gene expression in PC12 cells cultured in the RWV bioreactor using the microarray technique, and to utilize the information regarding gene expression to interpret putative specific functions that could be used as cellular biochemical markers in future research on the impact of environmental oxygen levels and cellular oxygen utilization under variable gravity conditions.

2. Materials and methods

2.1. Cell culture

Rat pheochromocytoma (PC12) cells were cultured in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum, 10% (v/v) horse serum, and 1% (v/v) penicillin-streptomycin-glutamine (all reagents; Gibco, Grand Island, NY). Cells were maintained in a humidified incubator with 5% CO_2 and 95% air mixture at 37 °C. For this experiment, Cytodex-3 microcarrier beads (Amersham Biosciences, Piscataway, NJ) were pretreated according to the manufacture's instructions to promote cell adhesion. Cell suspension (4×10^5 cells/ml) was first placed into 100 mm Petri dishes by adding beads (5 mg/ml). Cell/beads were combined to allow attachment of cells to beads for 24 h, and then, transferred into different culture environments: T-75 flasks that served as a static control and RWV (Synthecon, Houston, TX) bioreactors under simulated micro-g and unit-g as a dynamic control. The RWV bioreactors were oriented to expose microcarrier beads with non-confluent adherent PC12 cells to conditions of simulated micro-g (Fig. 1B) and normal unit-g by simply changing the position of the bioreactor (Fig. 1C). The RWVs were rotated at an initial speed of 15 rpm, and the speed was increased as the cell/bead aggregates enlarged. Culture medium was replaced every other day.

The microcarrier bead/cell suspensions were exposed to micro-g (M) for 1 or 4 days in the horizontal rotating RWV bioreactor (samples: M1 and M4) or to unit-g in the vertical rotating RWV bioreactor (samples: G1 and G4) and non-rotating static T-75 flasks (samples S1).

2.2. Microarrays

Total RNA isolated from rat PC12 cells released from microcarrier beads using RNeasy kit (Qiagen, Valencia, CA) according to the manufacturer's instructions was labeled as S1, M1, M4, G1, and G4, respectively (Fig. 1). The RNA quality and quantity were assessed by an ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE) and an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Palo Alto, CA). The rat 70-mer oligonucleotide library version 3.0 (27,342 optimized oligos) (Qiagen, Valencia, CA) was printed on aminosilane-coated slides (Cel Associates, Inc., Pearland, TX) using a high-speed robotic Omnigrid machine (GeneMachines, San Carlos, CA) with Stealth SMP3 pins (Telechem, Sunnyvale, CA).

Fluorescence-labeled cDNAs were synthesized from total RNA using an indirect amino allyl labeling method (Guo et al., 2004; Karyala et al., 2004; Sartor et al., 2004). The cDNA was

labeled with monofunctional reactive Cyanine-3 and Cyanine-5 dyes (Cy3 and Cy5; Amersham Biosciences, Piscataway, NJ). For the hybridization step, microarray slides were placed in humidified hybridization chambers (Corning, Acton, MA) in a water bath at 48 °C for 2 days. The slides were spun dried immediately after washing.

Imaging and data generation were carried out using a GenePix 4000A and GenePix 4000B (Axon Instruments, Union City, CA) and associated software from Axon Instruments. The microarray slides were scanned with dual lasers with excitation wavelength frequencies appropriate to elicit Cy3 and Cy5 specific fluorescence emission. Fluorescence images were captured in JPEG and TIFF files, and DNA spots were captured by the adaptive circle segmentation method.

2.3. Data analysis

The data representing raw spot intensities generated by GenePix® Pro version 5.0 software was analyzed to identify differentially expressed genes. Statistical analysis was performed by first normalizing by local regression per array, and then by fitting the following mixed effects linear model for each gene (Dudoit et al., 2002). $Y_{ijk} = \mu + A_i + S_j + C_k + \varepsilon_{ijk}$, where Y_{ijk} corresponds to the normalized log-intensity on the *i*th array, with the *j*th treatment combination, and labeled with the *k*th dye (Cy5 and Cy3). l is the overall mean log-intensity, A_i is the effect of the *i*th array, S_j is the effect of the *j*th treatment and C_k is the effect of the *k*th dye, ε_{ijk} is the random experimental error associated with Y_{ijk}. Assumptions about model parameters were the same as described by Wolfinger et al. (2001), with array effects assumed to be random, and treatment and dye effects assumed to be fixed.

After adjusting for array and dye effects, statistical significance of differential expression among RNA samples was assessed by calculating *p*-values, and estimates of fold-change were calculated. Multiple hypotheses testing adjustment was performed by calculating False Discovery Rates (FDR) for overall treatment effects (Benjamini and Hochberg, 1995; Reiner et al., 2003) and Bonferroni adjusted *p*-values for individual comparisons per gene. Data normalization and statistical analyses were performed using SAS statistical software package (SAS Institute Inc., Cary, North Carolina). Hierarchical clustering was performed using the average linkage clustering method (Eisen et al., 1998). Functional enrichment analysis was performed using Expression Analysis Systematic Explorer (EASE), and the EASE score and FDR were calculated (http://apps1.niaid.nih.gov/david). The EASE score is calculated as the upper bound of the distribution of Jackknife Fisher exact probabilities given the number of genes in the significant gene list (total and in the gene category), and the number of genes in the list of all genes analyzed (total and in the gene category) (Hosack et al., 2003).

3. Results and discussion

Using a FDR cut-off of 0.10 (10% false discovery rate), we found that 173 genes in PC12 cells cultured for 4 days under the simulated micro-g condition showed significant changes in gene expression compared with the unit-g control. Of these 173 genes, 104 genes revealed at least twofold change. Of these 104 genes, 65 were up-regulated and 39 were down-regulated.

Hierarchical clustering is a commonly used method to group genes according to their expression profiles. Results from clustering were used to produce a heat map showing global trends in expression differences. In Fig. 2, red indicates gene up-regulation between 1st and 2nd culture conditions of each column (M4/G4, M1/G1, G4/G1, and M4/M1), while green indicates down-regulation, and black means no change. Overall, a large number of genes were up- and down-regulated, and as expected, the number of genes that revealed at least a twofold change increased with the culture time (31 genes from day 1 culture and 104 genes from day 4 culture). Thus, it is evident that the gene changes progressed with the time duration of both

micro-g and unit-g exposure. In addition, as shown in Fig. 2, many genes follow a trend of increasing or decreasing from day 1 to day 4 between micro-g and unit-g exposure.

It is well established that oxygen-dependent cell function regulator genes can be used as markers of oxidative stress in PC12 cells (De Nigris et al., 2001). Several oxidation-sensitive transcription factors tested with a priori assumption, such as c-myc, hypoxia induced factor-2α (HIF-2α), and the peroxisome proliferator-activated receptor-γ (PPAR-γ)) (Haddad et al., 2000; De Nigris et al., 2001; Naranjo-Suarez et al., 2003) showed significant differential regulation, e.g., M4/G4: -17 ($p = 0.002$), 2.2 ($p = 0.027$), and -2.3 ($p = 0.024$) fold expression, respectively.

Subsequently, up- and down-regulated gene products were compared with functional assignment lists to determine which gene categories were significantly enriched with differentially expressed genes. This analysis was performed using Expression Analysis Systematic Explorer (EASE) in order to explore the putative biological role of any given group of genes, and the gene categories tested were the Molecular Function and Biological Process branches of the Gene Ontology database. Gene Ontology is a multi-organism, controlled vocabulary database containing three separate ontologies: biological process, molecular function, and cellular component, and it is commonly used for assessing the results of microarray analyses. The EASE score was calculated for each gene category in the significant list, and the gene categories were ranked by significance (Hosack et al., 2003).

3.1. Oxidoreductase responders

In the functional analysis result (Table 1), genes, involved in oxidoreductase activity category, which functions to catalyze oxidation-reduction reactions, were most significantly differentially expressed, as shown in Table 2. Of the 13 genes in oxidoreductase activity category, the largest changes in gene expression were observed for sepiapterin reductase (SPR), which catalyzes the terminal step in the biosynthesis of tetrahydrobiopterin (Fujimoto et al., 2003). This gene under micro-g compared to unit-g conditions was up-regulated by 3.6-fold after 4 days of culture. In a coordinate manner to SPR, dihydropteridine reductase (DHPR), which is involved in the reduction of dihydrobiopterin to tetrahydrobiopterin (Wilquet et al., 2004), revealed up-regulation by 2.2-fold. Catalase, which catalyzes the degradation of potentially injurious hydrogen peroxide, showed down-regulation by 2.4-fold. In Table 2, NADH dehydrogenase (ubiquinone) 1α subcomplex, 4 (NDUFA4), which transfers electrons from NADH to the respiratory chain, and ubiquinol-cytochrome *c* reductase binding protein (UQCRB), a nucleus-encoded component of ubiquinol-cytochrome *c* oxidoreductase (Complex III) in the mitochondrial respiratory chain are mouse and human orthologs, respectively.

3.2. Carbohydrate metabolism and cholesterol synthesis responders

Several genes, in the glucose and carbohydrate metabolism categories, revealed significant differential regulation under micro-g compared with unit-g (Table 3). Acetyl-co-enzyme A acetyltransferase 1 (ACAT1) involved in intra-cellular cholesterol metabolism and L-lactate dehydrogenase isoform A chain (LDH-A) involved in anaerobic glycolysis (as seen under conditions of muscle loading) were up-regulated, whereas glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a classical glycolytic protein that can serve as a general mediator of one or more pro-apoptotic pathways, and cytosolic NADP-dependent isocitrate dehydrogenase 1 (IDH1) whose activity is coordinately regulated with the cholesterol and fatty acid biosynthetic pathways and possibly the source for the cytosolic NADPH required by these pathways were down-regulated.

3.3. DNA repair and replication, cell proliferation and apoptosis responders

Differential expression levels of certain genes involved in DNA repair, DNA replication, and chromosome cycle, are listed in Table 4. Of those nine genes in the categories, ubiquitin-conjugating enzyme E2 C (UBE2C), a cell proliferation-related protein and pituitary tumor-transforming protein 1 (PTTG1), a novel anti-apoptotic oncogene expressed in most tumors (PC12 is a tumor cell line) which encodes a protein that is primarily involved in the regulation of sister chromatid separation during cell division showed significant up-regulations in gene expression after four-day culture under micro-g compared to unit-g by 13.6- and 8.8-fold, respectively. In Table 4, DNA repair protein rad51 homolog 1 (HSRAD51) which catalyzes strand exchanges between two homologous DNA molecules, ubiquitin-conjugating enzyme E2 C (UBE2C), a cell proliferation-related protein, and chromosome 11 open-reading frame 13 (C11ORF13), a proto-oncogene found in tumor cells are human orthologs.

3.4. Molecular transport and receptor responders

It was also observed that several oxidation-sensitive genetic markers significantly changed after 4 days of micro-g (Table 5). Genes participating in PC12 cell specific neurotransmitter (catecholamine) regulation - vesicular monoamine transporter 1 (Liu et al., 1992), synaptonemal complex protein SC65 (Chen et al., 1992), and chromogranin A precursor CGA (Kim et al., 2002) - were significantly up-regulated (3.1-, 2.2-, and 7.4-fold, respectively) by exposure to 4 days of micro-g. The adenosine A2A receptor gene (ADORA2A), another characteristic feature of PC12 cells known to be involved in regulation of blood vessel dilation in the adrenal medulla (Rudolphi et al., 1992), was up-regulated by 2.8-fold. The RAS-related protein (RAB-3A), an oxygen-sensitive neurotransmitter vesicular uptake regulator (Francis et al., 2002), also showed up-regulation by 2.2-fold. The anti-apoptotic heat shock protein 27 (HSPB1) (Benn et al., 2002) and a marker of cell stress-aging-neurodegeneration (Barford, 1996), protein phosphatase 1 were down-regulated by 4- and 2.9-fold, respectively.

3.5. Krebs cycle and oxidative phosphorylation responders

Many of these genes that revealed significant up- and down-regulation are associated with biological pathways such as citric acid cycle, electron-transport chain, and oxidative phosphorylation. For example, isocitrate dehydrogenase 1 (IDH1), which oxidizes isocitrate to the oxalosuccinate with the coupled reduction of NAD+ to NADH in the citric acid cycle (Krebs cycle), revealed significant regulation under micro-g. By conducting more experiments and identifying more gene pathways associated, it may possible to elucidate the fundamental mechanisms of micro-g effect on cellular physiology.

3.6. Shear stress responders

To uncouple the effect of shear stress from RWV-induced gene expression involved in oxidoreductase activity, the gene expression between unit-g RWV culture (G1 and G4) and static culture (S1) which satisfied the criteria for individual comparison ($p < 0.01$) were examined (Table 6). Of the 7 genes, only glucose-6-phosphate-1-dehydrogenase (G6PD) and isocitrate dehydrogenase 1 (IDH1) showed same directional changes at the comparisons of G1/S1, G4/G1, and G4/M4. Therefore this data implicate that these genes are likely responsive to shear stress, whereas others identified here were not significantly responsive to shear stress.

It is evident that the regulation of gene expression in PC12 cell lines is differentially and selectively responsive to gravity and to exposure duration. These results have let us to conclude that simulated micro-g conditions alter the expressions of several oxygen-sensitive transcription factors. It was observed that oxidoreductase activity was significantly changed under micro-g conditions. The potential contribution of gene expression microarray technology to expand our understanding of the gene expression under different culture conditions was

validated. Further, additional targeted experiments are needed to investigate the mechanisms of specific gene expressions by protein assays because it is possible that the shear force induced by RWV bioreactor might cause a different pattern of biological responses than obtained from a cell culture exposed in an Earth orbital micro-g laboratory setting (Hammond et al., 2000). Also gene responses might be expected to exhibit a temporal dynamic. Characterization of time-dependent responses will require a longer-term data series to confirm and extend the current microarray results with application of quantitative real-time polymerase chain reaction (QRTPCR).

The data obtained from this microarray experiment provides a valuable set of baseline gene response networks involved in an array of basic cellular responses to micro-g (e.g., pro- and anti-apoptosis, stress responses in carbohydrate and glucose metabolism, DNA repair and cell replications machinery, chemical and biological cell surface receptors and chemical neurotransmission, and ligand receptor processes). While some of these responses, which have been documented for PC12 cells exposed to micro-g conditions reflect systems or functions uniquely present in this PC12 cell line, the results establish a foundation for future exploration of micro-g effects combined with those of cell culture conditions (e.g., low and high ambient molecular oxygen or other nutrient conditions) with different cell lines (e.g., human umbilical vein endothelial cells). Future research on the human endothelial cell lines is of potentially greater direct relevance to the reality of astronauts, who will be exposed to both shorter and more prolonged micro-g environments.

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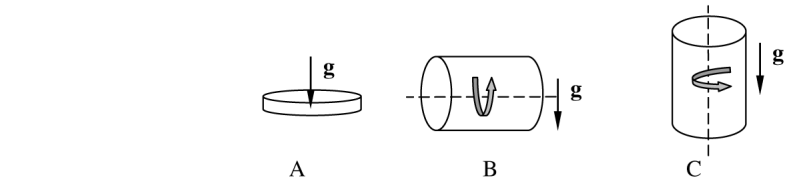

Condition / Culture time	Static Control (S)	Simulated micro-g (M)	Dynamic Control unit-g (G)
Day 1	S1	M1	G1
Day 4		M4	G4

Fig. 1.

Experimental design: static control (A), and slow turning lateral vessel type rotating wall vessels (RWV) in the simulated micro-g orientation (B) and in the unit-g orientation (C).

Fig. 2.

Hierarchical clusters diagram with gene Ensemble ID or names: Each column represents a single comparison and each row represents a single gene. Red color indicates up-regulation between the first and the second culture conditions [e.g., first column: M4 (micro-g for 4 day) versus G4 (unit-g for 4 day)], while green indicates down-regulation, and black means no change. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

Table 1

Functional analysis of most significantly changed gene categories (FDR[a]: < 0.05)

Gene category	List[b] Hits	List[c] Total	EASE score	GenBank/Refseq[d]
Oxidoreductase activity	13	54	6.90E-03	AF106860; D37920; J03481; J05031; L35317; M11670; M21018; M36410; M86870; X01964; X07467; NM_010886; NM_006294
Glucose metabolism	5	50	9.73E-03	AF106860; L22294; L36250; X01964; X07467
DNA repair	8	50	1.09E-02	AF290895; AJ222691; M60921; U73030; Y00047; NM_002875; NM_181799; NM_003475
Electron transport	7	50	1.12E-02	D37920; J03481; J05031; M11670; M21018; M86870; M91214
DNA replication and chromosome cycle	4	50	2.38E-02	AJ222691; U73030; U89282; Y00047
Carbohydrate metabolism	7	50	2.39E-02	AF106860; AJ243266; L22294; L35317; L36250; X01964; X07467
Response to stress	8	50	2.59E-02	AF290895; AJ222691; J02962; L35317; M11670; M60921; U73030; Y00047
Isomerase activity	4	54	4.18E-02	D14046; L36250; M21018; M86870

Genes can be assigned to more than one category.

[a] FDR, false discovery rate.

[b] List Hits, number of genes in the gene list that belong to the Gene category.

[c] List Total, number of genes in the gene list (Molecular function: 54, Biological process: 50).

[d] Orthologs.

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Table 2

Oxidoreductase activity category

Name	GenBank/Refseq[b]	*p*-value	FDR	Fold[a](M4/G4)
Glyceraldehyde-3-phosphate dehydrogenase (Gapdh)	AF106860	0.0002	0.0591	-2.06
Squalene monooxygenase	D37920	0.0010	0.0820	1.36
Dihydropteridine reductase (Dhpr)	J03481	0.0008	0.0773	2.20
Isovaleryl-CoA dehydrogenase	J05031	0.0005	0.0642	2.30
Isocitrate dehydrogenase 1	L35317	0.0002	0.0585	-2.18
Catalase	M11670	0.0001	0.0349	-2.36
Protein disulfide isomerase precursor (Pdi)	M21018	0.0009	0.0794	1.40
Sepiapterin reductase (Spr)	M36410	0.0001	0.0352	3.56
Protein disulfide isomerase A4 precursor (Erp72)	M86870	0.0003	0.0589	1.16
L-Lactate dehydrogenase A chain (Ldh-A)	X01964	0.0000	0.0004	1.56
Glucose-6-phosphate 1-dehydrogenase (G6Pd)	X07467	0.0000	0.0349	-1.58
(Mouse) NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 4 (NDUFA4)[b]	NM_010886	0.0016	0.0970	1.70
(Human) Ubiquinol-cytochrome *c* reductase binding protein (UQCRB)[b]	NM_006294	0.0005	0.0633	1.58

[a] Fold (M4/G4): fold-change in micro-g/unit-g for 4 day.

[b] Orthologs.

Table 3

Glucose and carbohydrate metabolism categories

Name	GenBank	*p*-value	FDR	Fold[a] (M4/G4)
Glyceraldehyde-3-phosphate dehydrogenase (Gapdh)	AF106860	0.0002	0.0591	-2.06
Mitochondrial aconitase (Nuclear Aco2 Gene)	AJ243266	0.0005	0.0630	1.40
Acetyl-coenzyme A acetyltransferase 1 (Acat1)	D00512	0.0001	0.0511	2.17
Isocitrate dehydrogenase 1	L35317	0.0002	0.0585	-2.18
Triosephosphate isomerase (Tpi1)	L36250	0.0012	0.0895	1.32
L-Lactate dehydrogenase A chain (Ldh-A)	X01964	0.0000	0.0004	1.56
Glucose-6-phosphate 1-dehydrogenase (G6Pd)	X07467	0.0000	0.0349	-1.58

[a]Fold (M4/G4): fold-change in micro-g/unit-g for 4 day.

Table 4

DNA repair, DNA replication, and chromosome cycle categories

Name	GenBank/Refseq[b]	*p*-value	FDR	Fold[a] (M4/G4)
X-Ray repair cross-complementing group 1 protein (Xrcc1)	AF290895	0.0005	0.0635	1.96
DNA polymerase delta catalytic subunit	AJ222691	0.0003	0.0595	2.32
B-cell translocation gene 2, anti- proliferative (Btg2)	M60921	0.0010	0.0815	2.82
Pituitary tumor-transforming protein 1(Pttg1)	U73030	0.0015	0.0971	8.80
Telomerase associated protein 1(Tep1)	U89282	0.0002	0.0651	-3.13
Proliferating cell nuclear antigen (Pcna)	Y00047	0.0006	0.0679	3.55
DNA repair protein rad51 homolog 1 (HSRAD51)[b]	NM_002875	0.0004	0.0610	7.63
Ubiquitin-conjugating enzyme E2 C (UBE2C)[b]	NM_181799	0.0000	0.0321	13.56
Chromosome 11 open-reading frame 13 (C11ORF13)[b]	NM_003475	0.0014	0.0950	1.41

[a] Fold (M4/G4): fold-change in micro-g/unit-g for 4 day.

[b] Human orthologs.

Table 5

Molecular transport and receptor responders

Name	GenBank	*p*-value	FDR	Fold[a] (M4/G4)
Chromogranin A precursor (Cga)	X06832	0.0006	0.0684	7.37
Vesicular monoamine transporter 1	M97380	0.0011	0.0867	3.13
Adenosine A2A receptor (Adora2a)	M91214	0.0009	0.0799	2.81
Ras-related protein (Rab-3 A)	X06889	0.0008	0.0780	2.18
Synaptonemal complex protein Sc65	X65454	0.0004	0.0630	2.16
Protein phosphatase 1, regulatory (inhibitor) subunit 14A (Ppp1r14a)	AF352572	0.0003	0.0632	-2.88
Heat shock 27 kDa protein (Hspb1)	M86389	0.0001	0.0378	-3.98

[a]Fold (M4/G4): fold-change in micro-g/unit-g for 4 day.

Table 6

Genes ($p < 0.01$, individual comparison criteria) in oxidoreductase activity category

Name	GenBank	Fold[a]		
		G1/S1	G4/G1	G4/M4
Isovaleryl-CoA dehydrogenase	J05031	-2.00	1.49	-2.30
Glyceraldehyde-3-phosphate dehydrogenase	AF106860	-2.13	1.67	2.06
Glucose-6-phosphate 1-dehydrogenase	X07467	1.38	1.85	1.58
Protein disulfide isomerase precursor	M21018	1.73	-2.11	-1.40
Isocitrate dehydrogenase 1	L35317	3.13	1.88	2.18
Squalene monooxygenase	D37920	1.33	-1.33	-1.36
Protein disulfide isomerase A4 precursor	M86870	4.48	-4.49	-1.16

[a] G, unit-g RWV; S, static control; M, micro-g RWV.